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CELLULAR AND MOLECULAR IMMUNOLOGY

Abul K. Abbas, MBBS

Professor and Chair
Department of Pathology
University of California–San Francisco School of Medicine
San Francisco, California

Andrew H. Lichtman, MD, PhD

Associate Professor of Pathology
Harvard Medical School
Brigham and Women's Hospital
Boston, Massachusetts

Jordan S. Pober, MD, PhD

Professor of Pathology, Immunobiology, and Dermatology
Yale University School of Medicine
New Haven, Connecticut

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AFFINITY CHROMATOGRAPHY

Affinity chromatography is used to purify an antigen or an antibody from a solution (Fig. A–4). An antibody is attached to a solid support, such as agarose beads packed into a column, either by direct coupling or indirectly, as described above for immunoprecipitation. A complex mixture of antigens is passed through the beads to allow the antigen that is recognized by the antibody to bind. Unbound molecules are washed away, and the bound antigen is eluted by changing the pH or by exposure to a chemical that breaks the antigen-antibody bonds. The same method may be used to purify antibodies from culture supernatants or natural fluids, such as serum, by first attaching the antigen to beads and passing the supernatants or serum through.

Labeling and Detection of Antigens in Cells and Tissues

Antibodies specific for antigens expressed on or in particular cell types are commonly used to identify these cells in tissues or cell suspensions and to separate these cells from mixed populations. In these methods, the antibody can be radiolabeled, enzyme linked, or most commonly, fluorescently labeled, and a detection system is used that can identify the bound antibody.

FLOW CYTOMETRY AND FLUORESCENCE-ACTIVATED CELL SORTING

The tissue lineage, maturation stage, or activation status of a cell can often be determined by analyzing the cell surface or intracellular expression of different molecules. This technique is commonly done by staining the cell with fluorescently labeled probes that are specific for those molecules and measuring the quantity of fluorescence emitted by the cell (Fig. A-5). The flow cytometer is a specialized instrument that can detect fluorescence on individual cells in a suspension and thereby determine the number of cells expressing the molecule to which a fluorescent probe binds. Suspensions of cells are incubated with fluorescently labeled probes, and the amount of probe bound by each cell in the population is measured by passing the cells one at a time through a fluorimeter with a laser-generated incident beam. The relative amounts of a particular molecule on different cell populations can be compared by staining each population with the same probe and determining the amount of fluorescence emitted. In preparation for flow cytometric analysis, cell suspensions are stained with the fluorescent probes of choice. Most often, these probes are fluorochrome-labeled antibodies specific for a cell surface molecule. Alternatively, cytoplasmic molecules can be stained by temporarily permeabilizing cells and permitting the labeled antibodies to enter through the plasma membrane. In addition to antibodies, various fluorescent indicators of cytoplasmic ion concentrations and reduction-oxidation potential can also be detected by flow cytometry. Cell cycle studies can be performed by flow cytometric analysis of cells stained with fluorescent DNA-binding probes such as

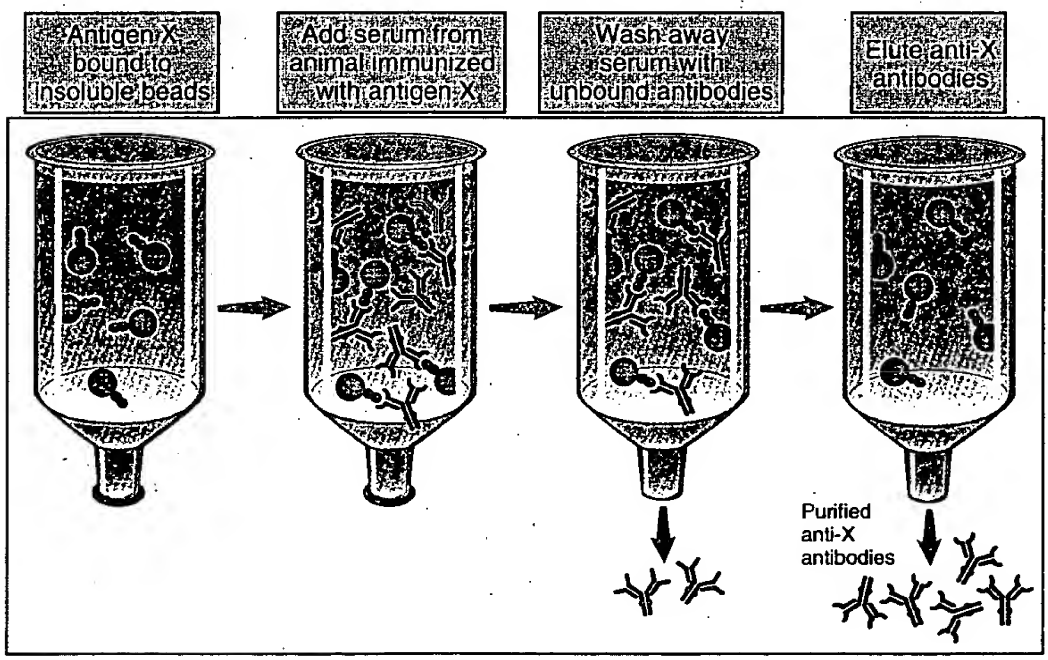

Figure A–4 Purification of antibody by affinity chromatography.
Antibodies specific for a particular antigen can be purified from a mixture of antibodies in serum or other solutions by passing the mixture through a matrix of insoluble beads to which the antigen is attached. The antigen-specific antibodies will bind to the beads, whereas the nonbonding antibodies are washed away. The bound antibodies can then be recovered (eluted) by changing the pH or ionic strength of the solution so that the affinity of antigen binding is lowered.

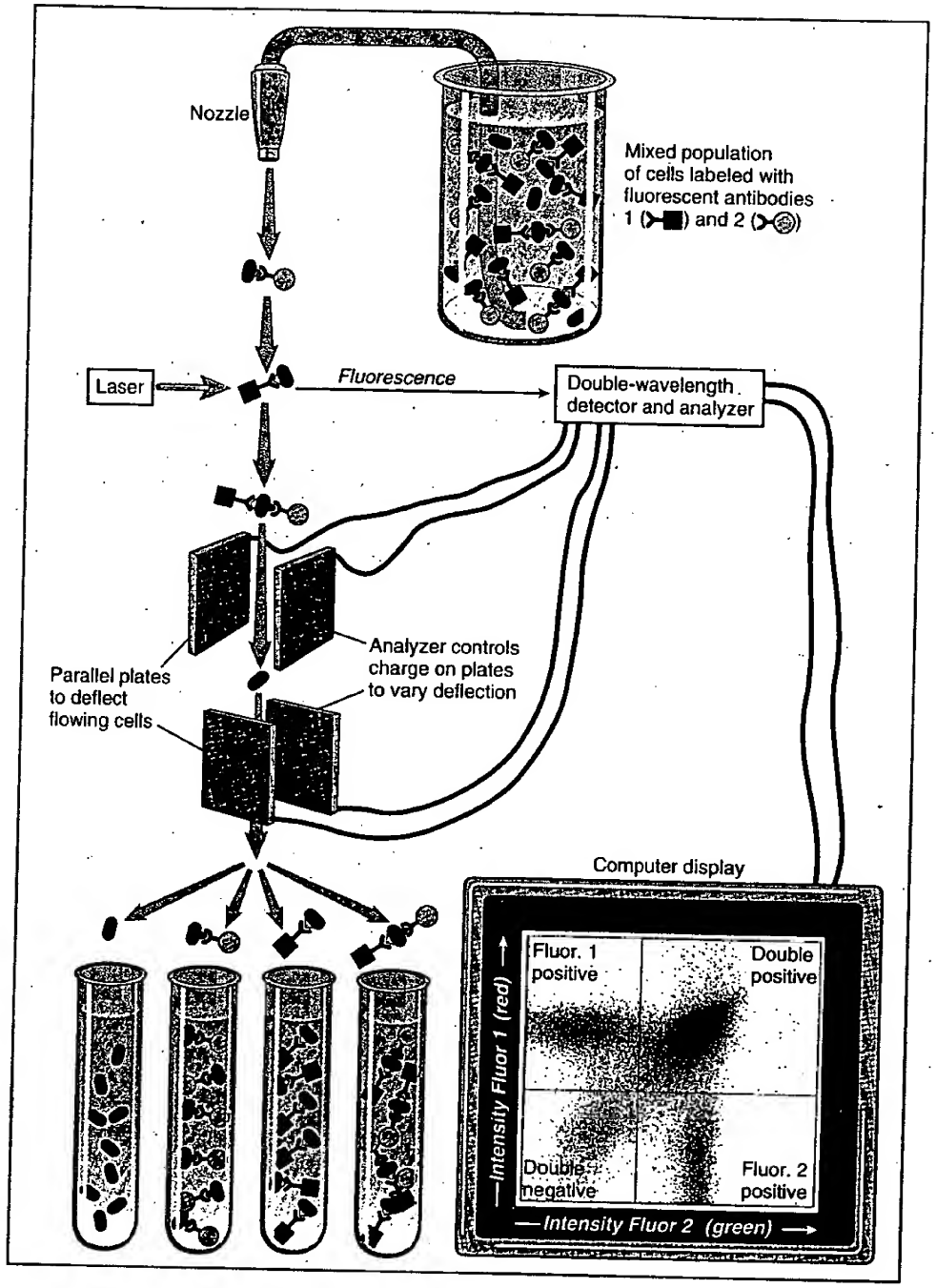

Figure A–5 Principle of flow cytometry and fluorescence-activated cell sorting.
The separation depicted here is based on two antigenic markers ("two-color sorting"). Modern instruments can routinely analyze and separate cell populations based on three or more different colored probes.

propidium iodide. Modern flow cytometers can routinely detect three or more different-colored fluorescent signals, each attached to a different antibody or other probe. This technique permits simultaneous analysis of the expression of many different combinations of molecules by a cell.

A fluorescence-activated cell sorter (FACS) is an adaptation of the flow cytometer that allows one to separate cell populations according to which and how much fluorescent probe they bind. This technique is accomplished by differentially deflecting the cells with electromagnetic fields whose strength and direction are varied according to the measured intensity of the fluorescence signal. A more rapid but less rigorous separation can be accomplished without a FACS by allowing cells to attach to antibodies bound to plates ("panning") or to magnetic beads that can be pulled out of solution by a strong magnet.

IMMUNOFLUORESCENCE AND IMMUNOHISTOCHEMISTRY

Antibodies can be used to identify the anatomic distribution of an antigen within a tissue or within compartments of a cell. To do so, the tissue or cell is